THE NITRITE-NITROSAMINE SITUATION: A REVIEW

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A. E. WASSERMAN,
Eastern Regional Research Center, Federal Research, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pa.

Dr. Wasserman was a member of USDA's Expert Panel on Nitrites-Nitrosamines from the Panel's inception in 1973 until its charter expired in September 1977.

In the Beginning

Nitrate in sea salt was used by earliest man.

AD

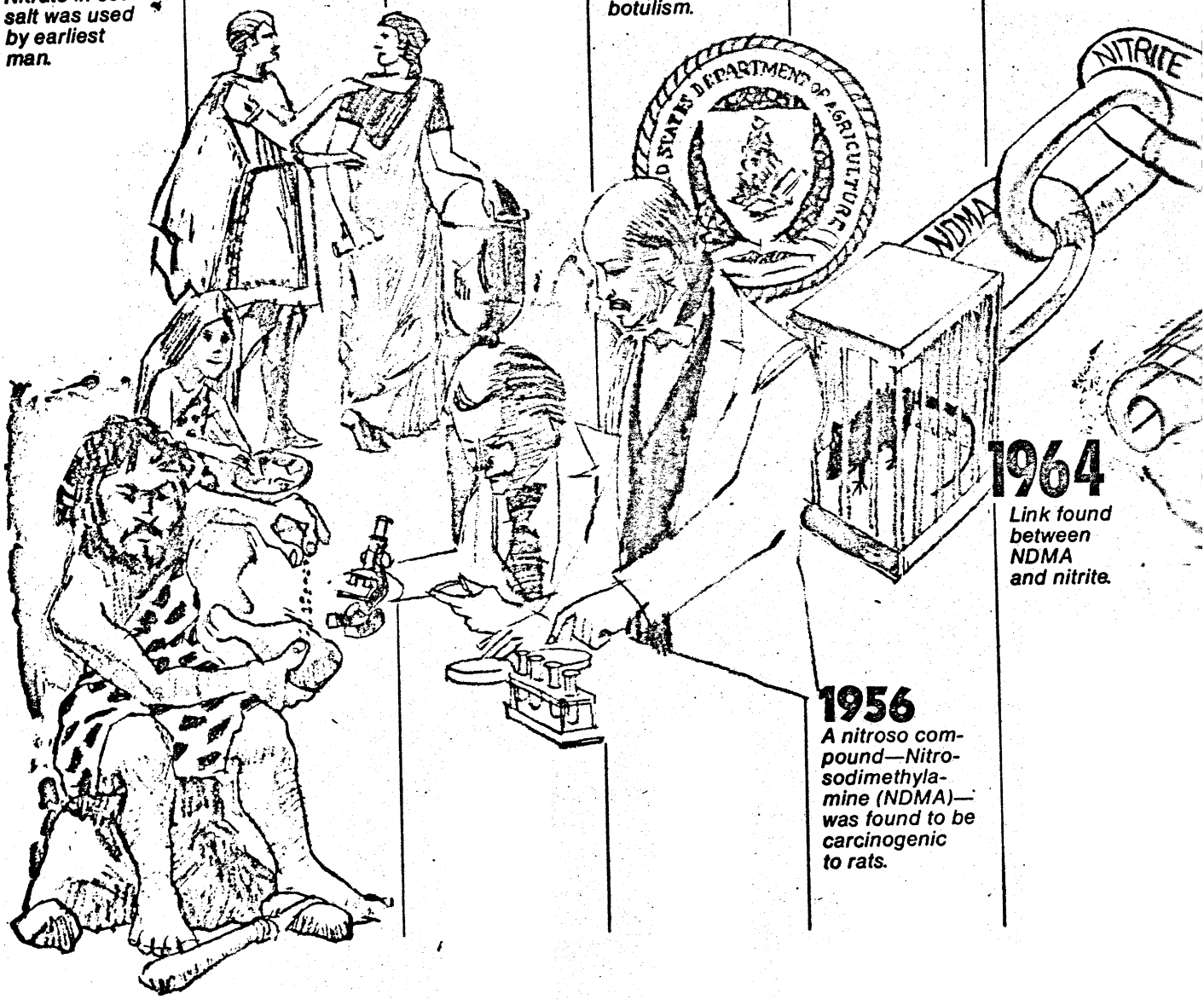
Nitrate was added to foods by the Greeks & Romans.

1890

Scientists found that bacteria converted nitrate into nitrite.

1925

USDA authorized use of nitrite to cure meat. Major step forward in preventing botulism.



1964

Link found between NDMA and nitrite.

1956

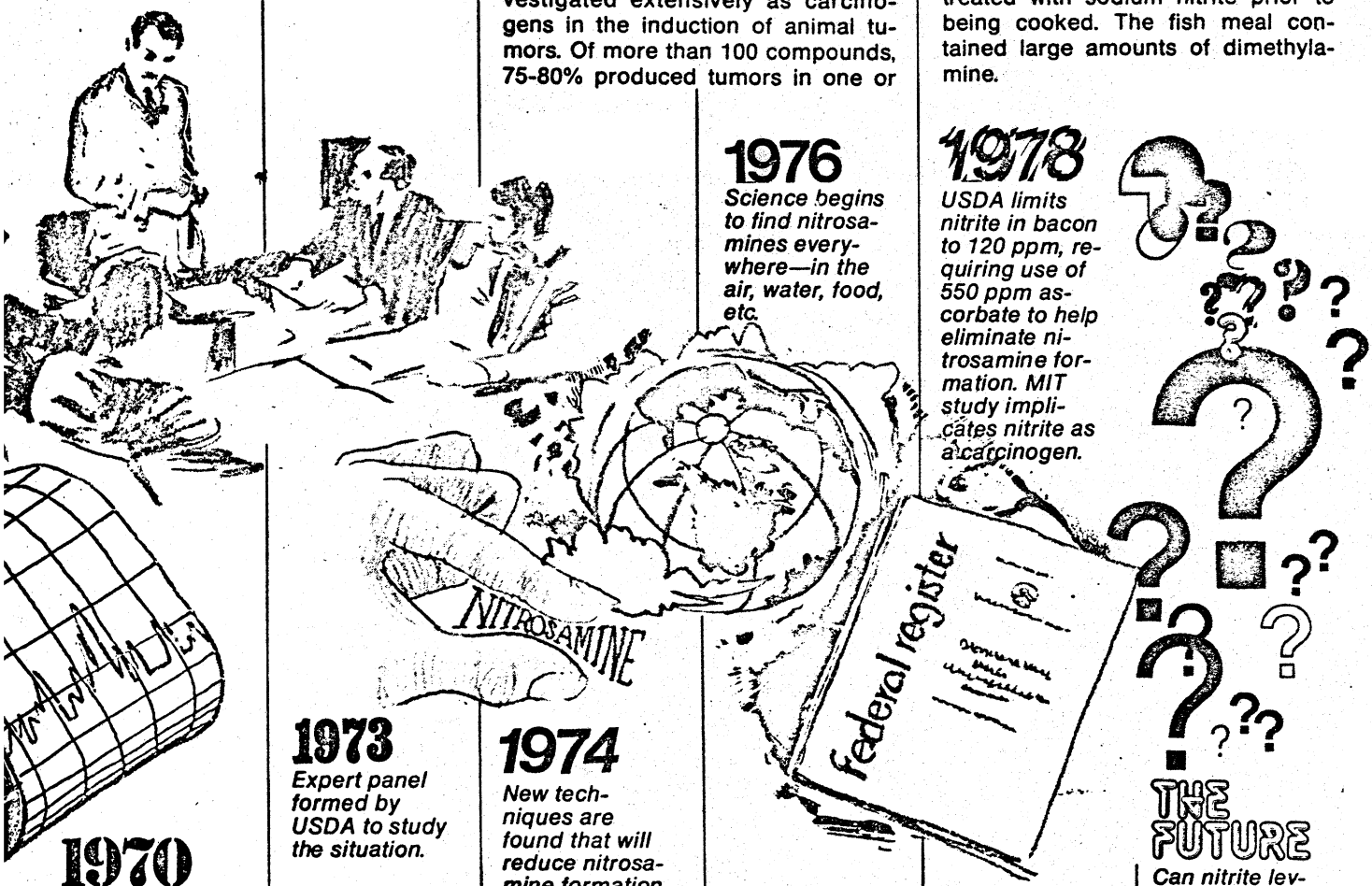
A nitroso compound—Nitrosodimethylamine (NDMA)—was found to be carcinogenic to rats.

Meat products cured with nitrite and/or nitrate and processed or cooked for consumption should not contain confirmable concentrations of nitrosamines. This is the policy of the U.S. Department of Agriculture as indicated by Dr. Robert Angelotti, Administrator of the Food Safety and Quality Service, in the October 18, 1977, issue of the Federal Register. A series of deadlines has been set for producers of bacon, ham, frankfurters, and other cured meat products to demonstrate that their products do not contain nitrosamines; a possible alternative is the banning of the use of nitrite and/or nitrate in meat processing.

Many N-nitroso compounds, including nitrosamines and nitrosamides, are carcinogens formed by the chemical reaction of nitric oxide (NO), which comes from nitrite or nitrate salts, with secondary amine (> NH) or amide (−CONH) groups. Nitrosamines were used in the past industrially for many years as solvents or intermediates in the chemical and pharmaceutical manufacturing industries. The synthesis of N-nitrosodimethylamine (NDMA) used to be used as a standard procedure in many college organic chemistry courses and the nitrosation of secondary amines was recommended as a qualitative procedure for differentiating the secondary from primary and tertiary amines.

In the early 1950's two British workmen, exposed to NDMA used as a solvent, developed cirrhosis of the liver. In 1954 Barnes and Magee demonstrated in rats that NDMA was toxic to the liver and was thus a hazardous substance. On continuing their studies, Magee and Barnes found that, at concentrations that were not hepatotoxic, NDMA produced liver tumors in the rats. Nitroso compounds were then investigated extensively as carcinogens in the induction of animal tumors. Of more than 100 compounds, 75-80% produced tumors in one or more species of animals in which they were tested. The tests showed species specificity, i.e., one animal species might develop tumors on exposure to a nitroso compound but another species would not, and organ specificity, i.e., certain nitroso compounds affected only one specific organ in a susceptible species. Since animals are susceptible to the carcinogenic activity of the nitroso compounds, humans might also be susceptible to the action of these compounds; however, no human cancers have been linked to or associated with exposure to nitrosamines. Even the two British workmen who developed cirrhosis of the liver did not develop cancer.

In the mid-1960's NDMA was discovered in large concentrations in the feed of sheep and mink that were suffering from liver disease; the source of the NDMA was traced to fish meal in the feed that had been treated with sodium nitrite prior to being cooked. The fish meal contained large amounts of dimethylamine.



1970
Sensitivity of instrumentation takes a quantum jump forward. Nitrosamines detected in nanogram quantities.

1973
Expert panel formed by USDA to study the situation.

1974
New techniques are found that will reduce nitrosamine formation in meat.

1976
Science begins to find nitrosamines everywhere—in the air, water, food, etc.

1978
USDA limits nitrite in bacon to 120 ppm, requiring use of 550 ppm ascorbate to help eliminate nitrosamine formation. MIT study implicates nitrite as a carcinogen.

THE FUTURE
Can nitrite levels be reduced further? Will a substitute for nitrite be found? Will new techniques prevent formation of nitrosamines? Will nitrite be banned?

NITRITE-NITROSAMINES...

Thousands of years

Nitrite has been used for curing and preserving meat and fish for thousands of years. In years gone by, nitrite was formed by the bacterial reduction of nitrate, since potassium nitrate ("saltpeter") was present as a contaminant in the sea salt used for food preservation. The use of nitrate was known to the Greeks and Romans, and saltpeter-preserved meat and game were staples through the Middle Ages and into the 19th century. After the reducing action of bacteria on nitrates was recognized (in the 1890's), nitrite was shown to produce the desired color and flavor of cured meats and, subsequently, to prevent the outgrowth of *Clostridium botulinum,* the organism producing the toxin that results in botulism food poison. In 1925 the Department of Agriculture approved the use of sodium nitrite in curing meats, and many producers used it in addition to, as well as in place of, potassium nitrate. It was advantageous to use nitrite because it afforded greater control over concentration and thus reduced concern for over- or undercuring the product, but a number of processors continue to use nitrate as a reservoir for nitrite.

Cured meat is not the only, or major, source of nitrite in the human diet. Saliva normally contains 8-12 ppm (mg/liter) nitrite, derived from bacterial reduction of nitrate, which is continually introduced into the saliva from the body. Potable water may contain up to 10 ppm nitrate legally, but many wells and streams contain greater concentrations. All plants contain nitrate, which is a source of their protein nitrogen. A number of leafy vegetables such as beets, lettuce, and spinach contain a thousand or more ppm nitrate as part of their normal composition. Within an hour after these vegetables are consumed, the concentration of nitrite in the saliva increases, reaching levels as high as several hundred ppm and remaining elevated for many hours. Nitrate has also been added to milk used for cheese making to prevent the formation of undesirable gas effects by clostridia. This is particularly true in some European countries; the use of nitrate in cheese has been prohibited in the United States.

Leafy vegetables contain thousands of ppm nitrate.

Amines: The other component

The other component in the reaction that produces nitroso compounds, consisting of amines or amides, also occurs naturally, either in the free state or as part of other compounds that can be broken down by the metabolic action of bacteria or animal tissue. Proteins and some amino acids produce secondary amines or amides; many amines, such as histamine, spermine, spermidine, sarcosine, tri-, di-, and mono-methylamine, are found in meat products.

Another source of amines is drugs. Many commonly used medications contain structures that yield nitrosatable amines. Piperazine, a de-worming agent, forms nitrosopiperazine; nitrosation of aminopyrine, chlorpromazine, oxytetracycline, or methadone produces NDMA; disulfiram yields N-nitrosodiethylamine. Other drugs have yielded nitrosamines when reacted with nitrite. In Germany the drug aminopyrine, which was used as freely as aspirin, was found to contain large quantities of NDMA and was banned from the market.

Sensitive analytical procedures

Developments in analytical methodology have had a great influence on the status of the nitrosamine situation. Prior to 1970 the principal analytical procedures involved thin-layer chromatography and color reagent sprays; however, interfering compounds gave false positive responses. Many studies of that period are suspect. About 1970 use of gas chromatography (gc) with special detectors, such as the alkali flame ionization, electron capture, and electrolytic conductivity, tremendously improved the specificity and sensitivity of the analytical procedures. Since the problem involves isolation and identification of trace quantities of nitrosamines (nanogram or less) extraction techniques were developed to separate these compounds quantitatively from accompanying natural compounds of similar structures and concentrate them for gc separation. However, even the special detectors at times responded to non-nitrosamines, giving false positive signals. Confirmation of the identity of the compound can be achieved with the mass spectrometer, although mass fragmentation patterns of non-nitrosamines that could not be distinguished from NDMA unless very high resolution was used have been reported. It is now generally accepted that authentic reports of nitrosamines must be confirmed by mass spectrometry, particularly for regulatory purposes.

The Thermal Energy Analyzer, an extremely sensitive instrument that measures picogram quantities of nitrosamines has been introduced recently. Unfortunately, the nitrosamine quantities that it can detect are so small that they are below the sensitivity of the mass spectrometer. This has now created a problem for regulatory purposes.

Widespread occurrence

Until recently, the principal, if not the only, source of human exposure to nitrosamines was believed to be the food ingested, but large amounts of nitrosamines in environmental situations have been reported, and research is determining further conditions in which nitrosamine formation can be expected.

In food, cured meat products have been the prime research target. The presence of nitrite, nitrate, and secondary amines appeared to offer an excellent opportunity for nitrosamine formation, and considerable research effort has been directed to these products. Cured ham, per se, has been found to contain little or no nitrosamines; however, when country cured hams were fried for six minutes on each side at 340°F, NDMA (30 to 60 ppb) was found in three of 25 hams studied. On the other hand, slices from these same hams fried for only three minutes on each side contained no nitrosamines.

The practice of storing dry premixes of cure ingredients such as nitrite, nitrate, salt, spices, sugar, and phosphates has resulted in the formation of nitrosopyrrolidine (attributable to paprika) and nitrosopiperidine (associated with components of pepper). The use of such premixes in making frankfurters and sausages could account for the nitrosamines found in three samples of frankfurters during a survey of more than 40 samples from seven major producers. Canadian investigators found this NDMA in Hungarian- and Italian-style sausage. Both Canada and the United States, and presumably other countries, have now banned the use of premixed cure salts.

Bacon is the most commonly used cured meat product in which nitrosamines have been found consistently. Raw bacon may contain nitrosoproline, which is a noncarcinogenic compound, but when it is fried for consumption (three minutes on each side at 340°F), nitrosopyrrolidine (NPyr) forms as the major (10-20 ppb) nitroso compound with low, variable concentrations of NDMA also present. Almost 100% of fried bacon samples tested contained nitrosamines. NPyr is formed mainly in

the fat or adipose tissue of the bacon; little or none is present in the lean tissue. Approximately 75-80% of the nitrosamines produced when bacon is fried have been found in the vapor over the cooking product, presumably due to steam distillation at the high temperatures used. Several studies suggest a time-temperature relationship; less NPyr is formed when bacon is cooked at lower temperatures or for shorter periods of time.

Sodium ascorbate (or sodium erythorbate), which had been used occasionally by some producers because it accelerated color formation, was found to react with the nitric oxide and reduce the amount of nitrosamine produced. Many producers are now voluntarily incorporating 550 ppm ascorbate or erythorbate in their cure formulations. Recently α-tocopherol has been shown to reduce the concentration of nitrosamines formed, and commercial scale studies are currently in progress to evaluate the use of these two inhibitors of nitrosamine formation.

Fish have also been investigated as a source of nitrosamines. Marine fish in particular contain high concentrations of trimethylamine, which is readily demethylated to dimethylamine and, on exposure to sodium nitrite, previously used as a preservative, forms NDMA. However, raw fish and fish treated with salt only were also found to contain NDMA. This was finally traced to the action of the bacteria, normally present on the fish, which reduced the nitrate in the salt to sufficient nitrite to form the nitrosamines.

Nitrosamines have been found in the air near industrial plants. At Baltimore, NDMA was detected near a process in which hydrazine was being produced. In a plant near Belle, West Virginia, in which dimethylamine was an intermediate in a process, NDMA was found in the air and waste water. The possibility of spontaneous nitrosamine formation in the atmosphere has been explored. Low concentrations of amines from natural sources can react with the NO_X in the air, particularly N_2O_3, to form nitrosamines. This could be catalyzed by acid conditions where the SO_2 concentration is high. However, because nitrosamines are UV-labile, large concentrations would not occur in the atmosphere, especially during the day.

Nitrosamines have been found in deionized water. This might impact on the processed food and drug industries, where such water could be used. Research is being carried out to determine the effect of the ion-exchange resins, bacterial influence, and other factors involved in this problem on the formation of nitrosamines.

Smoking tobacco is another potentially serious source of human exposure to nitrosamines. Not only is the smoker exposed but bystanders inhaling air contaminated with tobacco smoke also receive substantial doses of nitrosamines. Tobacco is rich in amines and compounds containing secondary amine structures and is also high in nitrate, particularly when cultivated with high-nitrate fertilizers. Some nitrosamines are found in the tobacco, others are formed in the smoke. Air samples from cocktail lounges and smoke-filled rooms have shown substantial levels of nitrosamines. No information is available about the carcinogenic effects of inhaled nitrosamines.

Recent area of concern

The most recent area of concern is *in vivo* nitrosation, the formation of nitrosamines in the stomach, the intestines, or the bladder from ingested or internally produced amines and nitrite. For the most part, this was inferred from animal studies in which tumors formed following ingestion of large doses of amines and nitrite; however, one study with a fistulated dog demonstrated the presence of nitrosamine in the stomach minutes after the amine and nitrite were introduced via a tube. Human experiments on the potential of *in vivo* nitrosation are difficult to perform, for obvious reasons. However, several studies that have been carried out in the last 10 years have strongly indicated that nitrosamines may form in the stomach, not only after eating foods containing precursors, but also under fasting conditions.

Control measures

Since the safety of cured meat products is the responsibility of the Department of Agriculture, then Secretary of Agriculture Butz established an Expert Panel in 1973 to study the use of nitrite and nitrate in cured meats and evaluate the nitrosamine situation. After listening to experts in various areas relating to the problem, and to consumer and industry representatives, the Panel presented recommendations to the Secretary in 1975. These included a ban on the use of nitrate on the grounds that this compound served only as a reservoir for nitrite, and the concentration of nitrite could be controlled more accurately by direct addition; various concentrations of nitrite, depending on the class of product, were proposed. Although the recommendations were published in the Federal Register and public comments were accepted, they were not issued as regulations. With the change in administration in 1977, several new members with strong consumer-advocate interests were added to the Panel and hearings were held more frequently in an effort to complete the study before the charter expired in September 1977. At the final meeting a new set of recommendations was proposed, very few with unanimous agreement of the Panel members. In general, the trend was to eliminate the use of nitrate, except in one or two classes of product in which safety and the need for the use of nitrate will have to be shown, and a reduction in the concentation of nitrite permitted to the levels that would prevent the germination of *Clostridium botulinum* spores.

Questions to be answered

For a true evaluation of the potential risk of cancer in humans from the consumption of processed meat or other foods with trace concentrations of nitrosamines, or as a result of the formation in the body following consumption of nitrate and/or amines, considerably more information is needed than is available at present. It is of primary importance to determine whether the low levels of nitrosamines that may occur in the diet will produce cancer in humans. Although tumors in animals can be produced by large doses of the precursors, many scientists believe the animal experiments do not serve as an adequate model, since the physiology and physical characteristics of the animals used differ so much from the human. Epidemiological studies are needed to determine whether people who have been exposed to nitrosamines in their work environment had a greater number of cancer incidents than the general population. This is a retrospective study; a prospective study would determine whether current exposure to nitrosamines or nitrite and secondary amines will lead to future cancer formation. The fate of the nitrosamine after it enters the body is also important. Is it destroyed by the body defenses? Is it converted to an active compound? Is the damage produced reversible? This information is not currently available. With the increased interest in this area and greater numbers of scientists engaged in research on nitrosamines, the answers to these questions, and many more, hopefully will not be long in forthcoming. FE